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The Development of Mold on Cold Storage Eggs and Methods of Control

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PART I.

ISOLATION AND IDENTIFICATION

INTRODUCTION

In 1926, 10,000,000 cases of eggs were placed in cold storage in the United States. This volume represented approximately 13 per cent of the entire annual crop. Unfortunately the preservation of eggs by cold, as practised today, is not the ideal to which the industry aspires, particularly for long periods of storage. High quality eggs are produced but some eggs become unmarketable during storage. There is a need for better storage conditions to eliminate spoilage by microorganisms and a fall in quality because of loss of water from the egg.

The process of holding foods at approximately freezing temperatures is a means of delaying decomposition or reducing decomposition to a minimum rather than a process of complete arrestment of microbiological and enzymatic spoilage. Microorganisms, particularly molds, will grow at exceedingly low temperatures provided they have sufficient moisture to germinate their spores and start the development of mycelium.

If the humidity of the storage room is high enough to form a microscopic film of moisture on the surfaces of the foods and their containers, then molds slowly but surely develop. If, on the other hand, the humidity of the storage room is kept at a point where the surfaces of the food and containers do not carry a microscopic film of moisture, then molds are prevented from growing, but the food gives up moisture to the air and shrinkage, with ultimate loss in quality of the food, results. In the operation of a cold storage plant, the humidities are, at present, kept at a point just below that which allows the development of microorganisms. This means that during storage a gradual loss of moisture from the food results. In eggs there is not only a loss in weight but the appearance of large air sacs, if the egg is held too long in storage.

The losses due to moldiness vary from season to season and from one plant to another. Many factors enter to determine the extent and degree of spoilage that occurs. Most of the factors under present operating conditions are uncontrollable; at least there is no practical means of controlling contaminations of eggs and egg cases. Moisture content of the storage rooms

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and storage temperatures cannot be varied sufficiently to be of any value in controlling the growth of the molds without causing greater injury to the product itself.

Molds have been reported as developing on a number of products in cold storage. The development of mold on meat in cold storage has been frequently observed. Monvoisin in 1922 (7) found *Cladosporium herbarum, Thamnidium elegans, Mucor mucedo, Rhizopus nigricans,* and *Penicillium glaucum.* Bidault in 1922 (2) found *Chaetostylum fresenii, Thamnidium elegans, Penicillium crustaceum, Hormodendron cladosporioides, Cladosporium herbarum, Stysanus stemonites, Botrytis micheli,* and some species of *Eubotrytis.* Wright in 1923 (14) found that "black spot" of meat on cold storage could be produced by a number of molds, including *Cladosporium herbarum, Mucor mucedo,* and *Penicillium glaucum.* In 1923 Yesair (15) found that *Penicillium expansum* was one of the common molds on the walls and equipment of meat packing houses. In 1927 Weston and Holman (13) demonstrated that a mold belonging to the genus Cladosporium was responsible for black discoloration of eggs. This discoloration, "black spot", was reproduced in eggs by treating clean eggs with spore suspensions of *Cladosporium herbarum.* Those workers also found that a species of Penicillium could produce a similar condition. In 1929 James and Swenson (6) in a similar study found two molds, a penicillium and a cladosporium.

To study the methods and procedures for mold control in the cold storage room, from a scientific standpoint, it was deemed essential to know exactly the species of molds encountered and the extent of their activity. Inasmuch as the literature does not reveal any such studies it was necessary to make a careful and extensive survey of the incidence and identity of the molds occurring on eggs, fillers, flats, and cases in the cold storage plant.

ISOLATION AND IDENTIFICATION OF MOLDS*

In order that a wide survey of the various species of molds be made, representative of the whole industry, eggs were obtained over a period of two seasons from plants in Chicago, Fort Wayne, Detroit, Lansing, Omaha, and Topeka. Fresh eggs from the College poultry plant and moldy eggs from cold storage were cultured. In addition to eggs, moldy fillers, flats and cases from the packing plants, and fillers, flats and cases from a manufacturer of the same were cultured for molds.

The isolations of molds were made by using scrapings from the surfaces of affected areas on the various materials examined. These scrapings were smeared on the surface of dextrose agar or bean-pod agar and incubated at room temperature until fruiting bodies of the molds appeared. The resulting mixed growths of molds were then purified by the dilution plate method. After the purity of each culture was definitely established, it was transferred to the proper media upon which identifications are based.

Except where indicated, the penicillia in this study were identified from Thom's "The Penicillia" (11). The genus Penicillium consists of a great many species which intergrade and which are exceedingly variable under different conditions of temperature, substratum, and manner of culture. The authorities on this genus, of whom Dr. Charles Thom of the United States Department of Agriculture is foremost, recognized many strains within each

*In the identification of the various fungi, assistance was received from Dr. E. A. Bessey, Experiment Station Botanist, who personally verified the identification of each culture.

species. A few species were identified by Dr. Thom. Where the description of an organism fell far outside of any standard description in Thom's key, it is designated by its culture number and the description is presented in detail.

Organisms belonging to the order Mucorales were identified from the descriptions of N. A. Naumov (9). Several forms of *Mucor racemosus* were isolated which fit this species most closely but are aberrant in some particular.

The identification of the aspergilli isolated correspond to the descriptions of Thom and Church (12).

A number of cultures were identified only as to genus. These belong to the *fungi imperfecti* and correspond to the description of the genus as found in standard works.

The descriptions of the aberrant species follow.

PENICILLIUM No. 30

Colony after four days on Czapek's agar light green with slight sterile border; reverse tan; drops absent. Colony after two weeks bright green with white margin about 2000 u broad; colony 4 cm. broad and about 1000 u deep; distinctly zonate; reverse cream to rose; agar faint rose color. Colony after 24 days 5.5 cm., bright green, velvety, deeply zonate at margin; reverse yellow-rose; agar rose-tan. Colony after six weeks dark green, deeply zonate at margin, with wide submerged margin; drops yellow; reverse dark red; agar dark red-brown; conidiophores slightly rough; metulae not divaricate, 9.44—11.8 u. Penicilli showing sterigmata and metulae in a compact brush or broom; sterigmata 5.9—(8.0)—10.6 u; conidia globose, smooth, 2.77—(3.0)—3.3 u; gelatin liquefied.

PENICILLIUM No. 36

Colony after four days on Czapek's agar pale green with white margin; drops absent; reverse light green. Colony after six weeks 8.5 cm. broad, velvety, thin felt, brown to gray-green, zonate at margin with wide submerged margin; white over-growth in center; reverse white to purple-black; conidiophores rough; metulae not divaricate, but penicilli subtended by one or more distant secondary divergent branches bearing secondary penicilli often monoverticillate; metulae 12.5—(13.0)—15. u; sterigmata 7.5—(12.0)—15.0 u; conidia smooth, globose 3.1—(3.58)—4.1 u; gelatin not liquefied.

PENICILLIUM No. 65

Colony after four days on Czapek's agar green with sterile white margin; drops absent; reverse tan-gray. Colony after 12 days 12.5 cm. x 5.5 cm., velvety, bright green with white margin 1000 u broad, zonate, about 900 u deep near edge; center about 2000 u deep; reverse orange yellow, zonate; agar colored red-brown; conidiophore erect, rough, asymmetrical, over 300 u long; metulae not divaricate 9.6—(9.65)—10.0 u; sterigmata 9.6—(9.65)—10.0 u; conidia globose, smooth 3.0—(3.7)—3.84 u; gelatin liquefied.

PENICILLIUM No. 84

Colony after four days on Czapek's agar green, white margin; reverse in shades of green; drops colorless. Colony after 26 days thin felt 3.5 cm. broad, floccose, olive-green, azonate; reverse in shades of green; agar uncolored; conidiophores rough; metulae not divaricate 10.0—(11.5)—15 u;

sterigmata 7.5—(10.5)—12.5 u; conidia smooth, globose 3.86—(4.0)—4.16 u; gelatin liquefied.

PENICILLIUM No. 102

Colony after four days on Czapek's agar light blue; drops colorless; reverse yellow-tan-rose. Colony after six weeks 8.5 cm., floccose, gray-green, faintly zonate at edge; wide submerged margin; drops colorless; reverse zones of rose red to cream; agar rose colored; conidiophores rough; metulae not divaricate 10—(11.5)—12.5 u; sterigmata 7.5—(9.0)—10.0 u; conidia globose, smooth, 3.3—(3.48)—3.6 u; gelatin liquefied.

PENICILLIUM No. 123

Colony after four days on Czapek's agar floccose, green with white margin; drops colorless; reverse light yellow. Colony after six weeks velvety, 4.5 cm. broad, dark green thin felt; wide submerged margin; slightly zonate at outer edge; drops colorless; reverse zones of pink to rose-tan; conidiophores rough; metulae not divaricate 12.5—(16.5)—10 u; sterigmata 12.5—(13.0)—15.0 u; conidia globose, smooth, 3.75—(3.83)—4.1 u; gelatin not liquefied.

PENICILLIUM No. 131.1*

Colony after two months on Czapek's agar 4 cm. long by 1—1.5 cm. broad, lanate, dark green with narrow white margin; much wrinkled; zonate in age; reverse mouse gray-violet, wrinkled, zonate; conidiophores short, smooth, 48—(60)—72 u, asymmetrical; metulae divaricate 8.4—(9.6)—10.8 u; sterigmata 4.8—(6.8)—8.4 u; 3-4-5 at end of metulae; conidia globose, smooth, 2.3—(3.1)—3.8 u; spore chains 36 u to 60 u.

PENICILLIUM No. 166

Colony after four days on Czapek's agar blue; margin very fuzzy; drops colorless; reverse light green. Colony after two weeks velvety, gray-blue with white margin 20000 u, azonate; colony 500 u deep, center umbonate; reverse cream; agar slightly cream-colored. Colony after six weeks dark blue-gray; zonate; reverse cream to rose shades; conidiophores slightly rough; metulae not divaricate 10—(11.5)—15 u; sterigmata 10—(11.9)—12.5 u; conidia globose, smooth, 2.77—(3.0)—3.5 u; gelatin liquefied.

PENICILLIUM No. 208

Colony after four days on Czapek's agar pale blue, very fuzzy with white margin; drops colorless; reverse white. Colony after twenty-four days floccose, blue-green; zonate at margin, reverse green-white; agar uncolored; metulae not divaricate 10—(10)—10 u; sterigmata 7.5—(9.5)—10 u; conidia globose, smooth, 3.1—(3.3)—3.57 u.

Mucor racemosus FRESENIUS

The three forms of this species that were most frequently isolated were the following. They do not seem to fit other described species but are quite distinct from one another.

*Dr. Charles Thom, of the United States Department of Agriculture, examined this culture and could not satisfy himself as to its specific identity; it seemed to conform to his 131.1 to which he had not assigned a specific name.

FORM NO. 1

Colony on dextrose agar after 10 days broadly spreading, mouse-gray; reverse light tan; colony 3.4 mm. high, very little branched; slender sporangiophores 1-3 mm. long; no stolons or rhizoids; sporangia 25-40 u, some sporangia encrusted; columella free, mostly oval, few conical, 19.2—(25.4)—33.6 u x 12.0—(20.7)—31.2 u; no chlamydospores; spores very slightly punctate, very slightly rough 4.0—(6.7)—11.5 x 3.6—(5.4)—9.8 u. This is a small form close to *Mucor racemosus,* but lacking chlamydospores, not cutinized, and not forming such tall sporangia as may occur in that species.

FORM NO. 2

Colony on dextrose agar after 10 days broadly spreading, gray; reverse light tan; colony 1 cm. high; sporangiophores about 2-6 mm., branched irregularly; sporangium membrane rough but not hairy, breaks into pieces, does not deliquesce; sporangia 21.6—(34.5)—55.2 u; columella slightly darkened; small collar around base of columella; collars vary in size; columella absent in lateral branches; columella shape pyramidal to ovoid, 20-30 x 14-20 u; chlamydospores numerous; spores elliptical, very slightly rough, variable in size 3.3—(6.6)—9.6 x 2.4—(4.9)—7.4 u.

FORM NO. 3

Colonies after three weeks tan; sporangia 33.6—(48)—84 u; sporangium membrane rough all over, breaks up, does not deliquesce; columella mainly panduriform, 19.2—(25.8)—32.4 x 12—(18.4)—19.2 u; pale brown; chlamydospores numerous. The columella frequently contains one, rarely 2 chlamydospores. The sporangia are in 2 layers near the base of the tall sporangiospores. There are many branches with numerous sporangia of various sizes. These small sporangia have a columella but it is smaller, almost spherical, about 7.2 x 7.2 u. The chlamydospores are quite numerous near the base of the sporangia. The lower mycelium is much roughened. Spores hyaline, light brown enmass; spores smooth, variable in size and shape, 3.6—(5.5)—12.0 x 3.6—(5.3)—9.6 u.

In all the studies which have been reported in the literature, there have been no attempts made to isolate and identify all the molds that might play a part in spoilage. All studies seem to have been limited largely to what might appear to be the predominating types.

In the studies herein presented, no attempt has been made to isolate all possible types and species. As previously stated, in order that a wide survey of the various species of molds be made representative of the whole industry, eggs were obtained over a period of two seasons from storage plants in Chicago, Fort Wayne, Detroit, Lansing, Omaha, and Topeka. The eggs obtained from these plants were rejects, visibly contaminated by molds.

In Table 1 a list of the molds isolated from the surface of the moldy eggs is presented. Although it was practically impossible to measure the extent of growth of each mold, still the number of isolations gives, at least, an estimate of the relative importance of each species.

A total of 400 isolations were made from the surface of moldy eggs. Of this total, 214 strains were penicillia, 50 strains were aspergilli and 80 strains were mucors. There was no relationship between place of storage or season and species.

After culturing the mold flora on the exterior of the eggs, the eggs were opened aseptically and cultures were made from the mold growths in the

TABLE 1. *Molds isolated from outer surface of moldy eggs.*

Name of mold	Number of isolations
Penicillium citreo-roseum Dierckx	12
Penicillium No. 102	2
Penicillium No. 36	5
Penicillium asperulum Bainier	10
Penicillium chloro-leucon Biourge	16
Penicillium ochro-chloron Biourge	4
Penicillium oxalicum Currie and Thom	10
Penicillium puberulum Bainier	36
Penicillium citrinum Thom	19
Penicillium janthinellum Biourge	16
Penicillium casei Staub	14
Penicillium crustosum Thom	16
Penicillium No. 131.1	17
Penicillium flavo-glaucum Biourge	10
Penicillium viridicatum Westling	6
Penicillium verrucosum Dierekx	12
Penicillium roqueforti Thom	9
Aspergillus flavus Lind	15
Aspergillus niger van Tieghem	35
Fusarium sp	5
Mucor racemosus Fresenius	50
Mucor lausannensis Lendner	30
Chaetomium globosum Knuze	24
Cephalosporium sp	15
Protabsidia Blakesleana Lendner	12
Actinomyces sp	Not counted

air sacs. The molds found growing on the inside of the egg were found, in all cases, on the surface of chorio-allantoid membrane, adjacent to the inner surface of the egg shell, in the air sac and on the inner surface of the shell. In no case did the mold seem to penetrate into the egg contents. In Table 2 is presented a list of molds cultured from the interior of the eggs showing macroscopic evidence of the growth of molds within the egg.

TABLE 2. *Molds isolated from the inside of eggs showing macroscopic evidence of mold inside the shell.*

Name of mold	Number of isolations
Penicillium citreo-roseum Dierckx	12
Penicillium No. 102	6
Penicillium No. 36	12
Penicillium asperulum Bainier	6
Penicillium chloro-leucon Biourge	34
Penicillium ochro-chloron Biourge	6
Penicillium oxalicum Currie and Thom	6
Penicillium puberulum Bainier	10
Penicillium citrinum Thom	6
Penicillium janthinellum Biourge	6
Penicillium casei Staub	2
Penicillium crustosum Thom	4
Penicillium No. 131.1	6
Penicillium flavo-glaucum Biourge	8
Penicillium viridicatum Westling	4
Penicillium verrucosum Dierckx	3
Penicillium roqueforti Thom	10
Penicillium No. 30	8
Penicillium No. 65	4
Penicillium No. 84	2
Penicillium No. 123	3
Penicillium No. 166	1
Penicillium No. 208	2
Penicillium Kapuscinskii Zaleski	3
Hormodendron sp	4

A total of 170 isolations was made of molds which occurred in the interior of moldy eggs. Of these, 166 strains were penicillia. Only one other genus, Hormodendron, was found.

The occurrence of molds on fresh eggs was also determined. Fresh eggs were obtained from the Michigan State College poultry plant. Attempts to isolate the molds by culturing dilution water used to wash the eggs were unsuccessful, revealing that the incidence of mold spores on the surface of eggs was low. That molds were on these eggs was demonstrated by placing the eggs aseptically into a sterile deep culture dish and pouring dextrose agar over the egg and incubating at room temperature. By this means the mold spores were able to germinate and grow. The molds isolated were similar to those isolated from moldy eggs.

Market eggs shipped to the cold storage plant for holding were also examined in the same manner as that used for fresh eggs. The same molds were isolated as in the case of fresh and moldy eggs. These eggs, however, showed a much higher incidence of molds than did the fresh eggs previously cited.

Moldy cases obtained from several cold storage houses were examined for molds. These cases showed visible evidence of mold development. The boxes were badly discolored by black and green mold spots. Cultures were taken from these areas. In Table 3 is presented a list of the molds isolated from moldy cases.

TABLE 3. *Molds isolated from moldy egg cases.*

Name of mold	Number of isolations
Penicillium citreo-roseum Dierckx	5
Penicillium No. 102	2
Penicillium asperulum Bainier	4
Penicillium oxalicum Currie and Thom	3
Penicillium puberulum Bainier	5
Penicillium Kapuscinskii Zaleski	4
Penicillium janthinellum Biourge	8
Penicillium citrinum Thom	3
Chaetomium globosum Kunze	4

Thirty-eight isolations were made. Thirty-four of the thirty-eight cultures were penicillia. Only one other organism, *Chaetomium globosum,* was isolated.

To determine the significance of new cases as a source of molds, a package of case boards was obtained directly from the manufacturer. To be certain that the molds represented initial contamination at the source, cultures were taken from the boards in the interior of the package. These boards, without exception, showed no visible growth of molds. Slivers of wood were cut from the boards, using aseptic precautions. These slivers were then cultured by embedding in dextrose agar. From five packages examined, only two molds were isolated, namely, *Mucor racemosus* and *Chaetomium globosum.* All the boards showed a heavy incidence of both molds. In the light of studies on eggs, the absence of penicillia and aspergilli shows that the new case material certainly is not a source of the objectionable penicillia. The data do show, however, that the new case material is heavily contaminated with molds.

Moldy fillers and flats were obtained from cold storage houses. These fillers and flats, like the moldy cases, showed marked evidence of mold forma-

tion. They were spotted with mold growth, generally black or green. In some instances the entire surface of the filler or flat was completely covered with mycelial growth and fruiting bodies. The isolations made from this material are presented in Table 4.

TABLE 4. *Molds isolated from moldy fillers and flats.*

Name of mold	Number of isolations
Penicillium citreo-roseum Dierckx	12
Penicillium No. 102	10
Penicillium No. 36	13
Penicillium asperulum Bainier	19
Penicillium chloro-leucon Biourge	25
Penicillium ochro-chloron Biourge	19
Penicillium oxalicum Currie and Thom	10
Penicillium puberulum Bainier	18
Penicillium citrinum Thom	10
Penicillium janthinellum Biourge	16
Penicillium casei Staub	4
Penicillium crustosum Thom	11
Penicillium flavo-glaucum Biourge	14
Penicillium viridicatum Westling	7
Penicillium verrucosum Dierckx	9
Penicillium roqueforti Thom	9
Penicillium No. 208	1
Penicillium corylophilum Dierckx	16
Aspergillus niger van Tieghem	5
Mucor racemosus Fresenius	27
Chaetomium globosum Kunze	4
Cephalosporium sp	7
Fusarium sp	3

A total of 269 strains of all molds was isolated from moldy fillers and flats. Two hundred and thirty-three of the isolations were penicillia.

To determine the occurrence of molds on fillers and flats, packages of new fillers and flats were obtained directly from the manufacturers. Representative culture samples were obtained by sampling aseptically from the center of each package. The isolations made were as follows:

Penicillium asperulum Bainier
Penicillium oxalicum Currie and Thom
Penicillium Kapuscinskii Zaleski
Aspergillus niger van Tieghem
Mucor racemosus Fresenius
Chaetomium globosum Kunze
Hormodendron sp.
Fusarium sp.

These data show that the molds on the new fillers and flats, although the incidence was low, are genera which may cause mold spoilage of eggs. The use of new fillers and flats would not appear to eliminate, so far as this source is concerned, the contamination of the eggs.

The close relationship of the penicillia found on the fillers, flats and outer shell of the eggs to the penicillia found inside of the egg is clearly shown in Table 5 in which the percentage of isolations for each organism has been determined. The occurrence, in general, indicates the ultimate passage of the organism through the shell into the egg. Although *Penicillium chloro-leucon* and *Penicillium puberulum* were found most frequently it would appear that many species of penicillia are able to enter the egg and cause spoilage.

Many other genera of molds, found on the egg surfaces and the fillers and flats, were apparently unable to penetrate the shell surface of the egg.

TABLE 5. *The percentage occurrence of various Penicillia species on the inside of the moldy egg in comparison with that of the outside of the moldy eggs, fillers and flats.*

Name of mold	Number of isolations		
	Inside of egg	Outside of egg	Fillers and flats
	(per cent)		
Penicillium citreo-roseum Dierckx	7.31	5.60	5.38
Penicillium No. 102	3.66	0.93	4.48
Penicillium No. 36	7.31	2.33	5.82
Penicillium asperulum Bainier	3.66	4.64	8.52
Penicillium chloro-leucon Biourge	20.73	7.45	11.21
Penicillium ocher-chloron Biourge	3.66	1.86	8.52
Penicillium oxalicum Currie and Thom	3.66	4.64	4.48
Penicillium puberulum Bainier	6.09	16.82	8.07
Penicillium citrinum Thom	3.66	8.87	4.48
Penicillium janthinellum Biourge	3.66	7.45	7.17
Penicillium casei Staub	1.21	6.54	1.79
Penicillium crustosum Thom	2.44	7.45	4.93
Penicillium flavo-glaucum Biourge	4.87	4.64	6.27
Penicillium viridicatum Westling	2.44	2.80	3.13
Penicillium verrucosum Dierckx	1.82	5.60	4.03
Penicillium roqueforti Thom	6.09	4.20	4.03
Penicillium No. 208	0	0	0.44
Penicillium corylophilum Dierckx	0	0	7.17
Penicillium No. 30	4.87	0	0
Penicillium No. 65	2.44	0	0
Penicillium No. 84	1.21	0	0
Penicillium No. 123	1.82	0	0
Penicillium No. 131.3	3.66	7.94	0
Penicillium No. 166	0.61	0	0
Penicillium Kapuscinskii Zaleski	1.84	0	0

This is clearly shown in Table 6. These data show the frequency of appearance of the mucors. These molds do not cause any apparent damage to the egg quality. They are exceedingly important because these organisms are the "fuzz" molds that are the first indications of mold formation on storage eggs. They do reduce the sales value of the eggs if they are allowed to develop beyond the "fuzz" stage. They are excellent danger indicators of reduced egg quality and have been used as such by the trade. Frequently the humidity of the storage room has been raised until these organisms appear.

TABLE 6. *The comparison of the various molds on moldy eggs and moldy egg containers.*

Name of mold	Number of isolations			
	Outside of egg	Inside of egg	Egg cases	Fillers and flats
Aspergillus niger van Tieghem	35	0	0	0
Aspergillus flavus Link	15	0	0	0
Fusarium sp	5	0	0	3
Mucor racemosus Fresenius	50	0	0	27
Mucor lausannensis Lendner	30	0	0	0
Chaetomium globosum Kunze	24	0	4	4
Cephalosporium sp	15	0	0	7
Protabsidia Blakesleana Lendner	12	0	0	0
Hormodendron sp	0	4	0	0
All *penicillia* species	214	150	34	223

To show the ability of penicillia to penetrate the shells, eggs showing no evidence of mold growth were washed with mercuric chloride and sterile water and inoculated with the spores by placing them in the air sac without

contamination of the outer shell surfaces. This was done by injecting the spores directly into the air sac with a hypodermic needle. After the injection was made, the hole made by the needle was covered with paraffin. After 14 months there was no evidence of mold growth on the outside of the eggshells. Upon opening the eggs, the mold was found to be alive. The mold had grown extensively on both the outer surface of the membrane and the inner surface of the shell. The molds had not penetrated either the shell or the membrane.

The same penicillia used for injection of air sacs were also placed on the unbroken surface of fresh eggs. These eggs, when examined, showed the presence of the mold in the air sacs of the eggs. Several cases of eggs in which the eggs were sprayed with a mixture of penicillia spores showed infection in approximately 90 per cent after 12 months storage under normal storage conditions.

SUMMARY

All the fungi obtained from eggs and egg materials in this study belong to the large group of organisms which live primarily as saprophytes in the soil and on decaying vegetative material in the soil. They are, however, ubiquitous. They are present in the nest and on the feathers of the hen. They pass unharmed through the intestinal tract of chickens. Therefore, it is evident that eggs are laid in an environment heavily seeded with these spores.

This study shows that the packing materials also contain these organisms. They are present not only on used materials but on new cases, fillers, and flats. These organisms likewise inhabit the packing and storage houses, particularly when the humidity is high enough to support their growth.

Although the environment and the shells of both fresh and moldy eggs contain a large variety of molds, not all of them penetrate the interior of the egg under cold storage conditions. Only two genera were included in those isolated from the interior of the shell. These consisted of 25 species of penicillia and four strains of Hormodendron.

Although the genus Penicillium was isolated most frequently in these experiments and was probably responsible for the contamination, no one species can be incriminated as a causative agent for the mold spoilage.

All the evidence of this study shows definitely that the mold found on moldy eggs comes from contamination from such sources as the egg itself and the handling and packing, both before and after reaching the cold storage plant.

With conditions conducive to the growth of molds in the cold storage rooms, it would appear impossible to prevent mold formation irrespective of the sanitary precautions exercised in the handling and packing of the eggs. This statement should not be interpreted to mean that sanitation need not be exercised, because the extent and degree of contamination can be materially reduced by sanitary precautions.

PART II.

LABORATORY STUDIES ON MYCOSTATIC AGENTS

INTRODUCTION

The presence of ubiquitous molds on the eggs, fillers, flats, case and in the atmosphere of the cold storage room indicates that irrespective of sanitary precautions in the packing and handling of eggs, little improvement can be expected in the control of mold. The writers believe that the control of molds lies primarily in the prevention of growth by introducing into the egg containers or upon the egg surfaces, chemical agents which will prevent the development of the existing mold spores without imparting any undesirable flavors or odors to the eggs and not impairing their food value in any manner.

Chemical agents have been previously tried with some success. Ewell in 1936 (4) and in 1938 (5) reported that 1.5 p.p.m. of ozone in the aisles of cold storage rooms provides ample protection against molds at a relative humidity of approximately 90 per cent. However, the literature (1) reveals some controversy as to the value of ozone when used as a mold preventive. A plant operator stated that after 48 hours in an atmosphere containing 5 p.p.m. of ozone, molds on eggs will "actually disappear as though they had evaporated". Another plant operator stated, "It cannot be done".

Probably one of the greatest obstacles in the use of ozone is the cost of installing and maintaining ducts. Most plants have considerable difficulty from the corrosive action of ozone on the ducts (1). In many cold storage houses, eggs are stored in the same room as food products that are affected adversely by ozone. Under such conditions ozone could not be used.

Moran in 1938 (8) found that the use of carbon dioxide would retard mold growth and permit accordingly higher relative humidities. He found that at a relative humidity of 85 per cent at 32° F. the air containing 2.5 per cent carbon dioxide was necessary to prevent the development of molds. At 32° F., 60-per cent carbon dioxide was necessary to suppress molds if the atmosphere was saturated with moisture. At that concentration of carbon dioxide, however, the whites of the eggs assumed a watery consistency.

Pennington (10) states that it is difficult to maintain definite concentrations of gas in rooms not adapted to gas storage and that the average cold storage rooms are not designed for gas storage.

Attempts have been made to control mold contamination by ridding the storage room of molds and placing antiseptic preparations on the walls. Some plants practice routinely a fumigation of the room each year prior to the storage of eggs. Sometimes this fumigation is supplemented by washing or spraying the walls with a solution of sodium hypochlorite, sodium ortho-phenyl phenate, phenyl mercuric nitrate or other disinfectants or antiseptics. There is no question but that such practice does lessen materially the contamination of the room but such practice has little or no effect upon the interior of the egg package or the egg. In an earlier part of this bulletin, it was definitely demonstrated that the eggs and the egg containers carry the undesirable

molds in varying numbers, and obviously it is those organisms that affect the eggs if moisture conditions are conducive to their growth. Any method of control to be successful at relatively high humidities must prevent the development of the organism on the egg and the container area which is directly in contact with the egg. The research work reported in this bulletin was planned with that objective as a goal.

Two types of disinfecting agents were tested, first, agents with marked mycostatic* properties with little or no vapor pressure, which act by direct contact with the mold spore or mycelium and, second, agents with marked mycostatic properties with vapor pressures sufficient to give off, slowly and continuously, mycostatic vapor inhibitory to the mold spore or mycelium.

The first type of compound could be incorporated into the fillers and flats. Thus the egg would be enveloped in a cell, the walls of which would prevent the development of molds. Any mold development that might occur within or outside the individual egg would at least be localized to the particular egg affected. If eggs with relatively low initial contamination were stored such procedure would lessen the amount of spread of mold from one cell to another and would materially extend the time of storage before the appearance of mold mycelia.

The second type of compound could also be incorporated into the fillers and flats. These compounds, owing to this high vapor pressure, would not only inclose the egg in a cell whose walls were mycostatic, but would also, theoretically, bathe the egg continuously in a mycostatic vapor. Such preparations would thus completely block any possible mold development on the egg. Theoretically such compounds would be ideal, provided they had no effect upon the egg quality.

LABORATORY TESTS ON MYCOSTATIC AGENTS

After a number of procedures had been tried with varying degrees of success, the following method was finally adopted for measuring the mycostatic powers of the selected compounds: The mycostatic agent was mixed in varying concentrations with a suitable sterile nutrient medium, such as bean-pod or dextrose agar. The medium containing the mycostatic agents were poured into sterile Petri dishes and the agar was allowed to harden. Spores of the various molds were placed on the surface of the medium in a circumscribed area. Growth on the plates was recorded quantitatively by the extent and degree of development.

Tests on Direct Contact Mycostatic Agents

To limit the amount of work, only three organisms were used in the early studies. These were, of necessity, more or less selected at random from the cultures which were isolated from eggs and egg containers. The three organisms selected were *Penicillium puberulum, Aspergillus niger,* and an unidentified species of *Alternaria.*

A large number of contact mycostatic agents was tested but only a few agents will be discussed because most of them were found to be exceedingly low in their mycostatic activity or they had other properties which would prevent their use.

*The words "mycostatic" and "mycostat" are used in this bulletin because the compounds are not used as fungicides. The use of the term fungicide conveys a wrong impression of the action obtained.

TABLE 7. *The mycostatic properties of three compounds commonly used in food preservation.*

Mycostat and test organism	Per cent concentration of mycostat										
	1.0	.9	.8	.7	.6	.5	.4	.3	.2	.1	Control
Boric acid:											
Alternaria sp.	−	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+
Aspergillus niger	−	−	2+	2+	2+	2+	2+	2+	2+	2+	3+
Penicillium puberulum	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+
Sodium borate:											
Alternaria sp.	−	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+
Aspergillus niger	−	−	−	−	−	−	−	−	−	−	3+
Penicillium puberulum	−	−	−	−	−	−	−	−	−	2+	3+
Sodium benzoate:											
Alternaria sp.	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+
Aspergillus niger	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+
Penicillium puberulum	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+

3+ excellent growth; 2+ good growth; − no growth.

The first series of compounds consisted of products with relatively low toxicities, which would not be objectionable in surface contact of food products. Of this group, three are presented, namely sodium borate, boric acid, and sodium benzoate. The data are presented in Table 7.

Sodium benzoate even in 1-per cent concentrations failed to have any inhibitory effect upon the three molds tested. Boric acid had only a slight action on *Alternaria sp.* and *Aspergillus niger*. Sodium borate caused inhibition in dilutions as low as 0.2 per cent on *Penicillia puberulum* and *Aspergillus niger* but it took a 1-per cent concentration to inhibit *Alternaria sp.*

Since the three genera of molds tested varied so widely in their resistance, another series of molds was tested, this time using only various strains and species of penicillia. In Table 8, the data for fourteen strains and species of penicillia are presented on sodium borate. It will be observed that wide

TABLE 8. *The resistance of 14 cultures of penicillia to sodium borate.*

Organism	Per cent concentration of sodium borate									
	.8	.7	.6	.5	.4	.3	.2	.1	.02	Control
P. puberulum, No. 1	−	−	−	−	−	−	−	+	+	3+
P. puberulum, No. 2	−	−	−	+	+	+	+	+	+	3+
P. puberulum, No. 3	−	−	−	−	+	+	+	+	+	3+
P. puberulum, No. 4	−	−	+	+	+	+	+	+	+	3+
P. roqueforti	−	−	−	−	−	−	+	+	+	3+
P. verrucosum	−	−	−	−	+	+	+	+	+	3+
P. casei	−	−	−	+	+	+	+	+	+	3+
P. citrinum	−	−	−	+	+	+	+	+	+	3+
P. crustosum	−	−	−	−	+	+	+	+	+	3+
P. oxalicum	−	−	−	−	+	+	+	+	+	3+
P. 131.1	−	−	−	−	+	+	+	+	+	3+
P. flavo-glaucum	−	−	−	−	−	−	−	+	+	3+
P. janthinellum	−	−	−	+	+	+	+	+	+	3+
P. viridicatum	−	−	−	−	+	+	+	+	+	3+

3+ excellent growth; + fair growth; − no growth.

variations in resistance occurred. In the four strains of *Penicillium puberulum,* one strain (No. 4) was inhibited at 0.7 per cent, whereas strain No. 1 was inhibited at 0.2 per cent.

Of the three compounds reported, only sodium borate showed any value and, as indicated in Table 8, such marked variations occurred in the various strains of penicillia, coupled with the lack of inhibition of *Alternaria sp.* demonstrated, that the use of this compound appeared to have little value.

The lack of inhibition by those compounds is largely due to the pH of the test material. Both sodium benzoate and sodium borate are much more effective in an acid medium; however, the tests presented were made at a pH of approximately 7, which more nearly simulates the pH that would be encountered in actual practice.

The heavy metals, particularly silver, mercury, and copper have considerable mycostatic properties. Copper salts were selected for test because they are widely used in the control of fungus diseases of plants. Although copper is not permissible in foods, still it was hoped that dilute solutions might show sufficient mycostatic action to allow its use in fillers and flats. This would in no way affect the eggs.

The following copper salts were tested; cupric nitrate, cupric sulphate, cupric ammonium sulphate, cupric ammonium chloride, cupric acetate, and cupric chloride. These compounds were tested against *Penicillium puberulum, Aspergillus niger* and *Alternaria sp.* The data are presented in Table 9.

TABLE 9. *The mycostatic properties of six copper compounds.*

Mycostat	Test organism	Per cent concentration of copper compounds							
		1.0	0.9	0.8	0.7	0.6	0.5	0.1	Control
Cupric nitrate:									
	Alternaria sp.	−	−	−	−	−	−	2+	3+
	Aspergillus niger	2+	2+	2+	2+	2+	2+	2+	3+
	Penicillium puberulum	−	−	−	−	−	−	2+	3+
Cupric sulphate:									
	Alternaria sp.	−	−	−	+	+	+	2+	3+
	Aspergillus niger	−	−	−	−	−	−	2+	3+
	Penicillium puberulum	−	?	?	?	?	2+	2+	3+
Cupric ammonium sulphate:									
	Alternaria sp.	−	−	−	−	−	−	2+	3+
	Aspergillus niger	2+	2+	2+	2+	2+	2+	2+	3+
	Penicillium puberulum	−	−	−	−	−	−	2+	3+
Cupric ammonium chloride:									
	Alternaria sp.	−	−	−	−	−	−	2+	3+
	Aspergillus niger	2+	2+	2+	2+	2+	2+	2+	3+
	Penicillium puberulum	−	−	−	−	−	−	2+	3+
Cupric acetate:									
	Alternaria sp.	−	−	−	−	−	−	2+	3+
	Aspergillus niger	−	−	−	−	−	−	2+	3+
	Penicillium puberulum	−	−	−	−	−	−	2+	3+
Cupric chloride:									
	Alternaria sp.	−	−	−	−	−	−	+	3+
	Aspergillus niger	2+	2+	2+	2+	2+	2+	2+	3+
	Penicillium puberulum	−	−	−	−	−	−	−	3+

3+ excellent growth; 2+ good growth; − no growth.

TABLE 10. *The mycostatic powers of five phenol derivatives on four genera of molds.*

Mycostat	Test organism	Per cent concentration of mycostat													
		.2	.04	.03	.02	.009	.008	.007	.006	.005	.004	.003	.002	.001	Control
Sodium orthophenylphenate (Dowicide A)	*Aspergillus niger*	–	–	–	–	?	?	?	?	3+	3+	3+	3+	3+	3+
	Penicillium puberulum	–	–	–	–	–	3+	3+	3+	3+	3+	3+	3+	3+	3+
	Chaetomium globosum	–	–	–	–	–	–	+	+	+	+	2+	3+	3+	3+
	Mucor racemosus	–	–	–	–	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Sodium 2, 4, 5 trichlorophenate (Dowicide B)	*Aspergillus niger*	–	–	–	–	–	–	–	–	–	–	–	–	–	3+
	Penicillium puberulum	–	–	–	–	–	–	–	–	–	–	–	–	3+	3+
	Chaetomium globosum	–	–	–	–	–	–	–	–	–	–	–	–	–	3+
	Mucor racemosus	–	–	–	–	–	–	–	–	–	–	3+	3+	3+	3+
Sodium 2 chloroorthophenylphenate (Dowicide C)	*Aspergillus niger*	–	–	–	–	–	–	–	–	?	3+	3+	3+	3+	3+
	Penicillium puberulum	–	–	–	–	–	–	–	–	–	3+	3+	3+	3+	3+
	Chaetomium globosum	–	–	–	–	–	–	–	–	–	+	2+	2+	3+	3+
	Mucor racemosus	–	–	–	–	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Sodium 2, 4, 5, 6 tetrachlorophenate (Dowicide F)	*Aspergillus niger*	–	–	–	–	–	–	–	–	–	–	–	–	3+	3+
	Penicillium puberulum	–	–	–	–	–	–	–	–	–	–	3+	3+	3+	3+
	Chaetomium globosum	–	–	–	–	–	–	–	–	3+	3+	3+	3+	3+	3+
	Mucor racemosus	–	–	–	–	–	3+	3+	3+	3+	3+	3+	3+	3+	3+
Sodium pentachlorophenate (Dowicide G)	*Aspergillus niger*	–	–	–	–	–	–	–	–	–	–	–	3+	3+	3+
	Penicillium puberulum	–	–	–	–	–	–	–	–	–	2+	3+	3+	3+	3+
	Chaetomium globosum	–	–	–	–	–	–	–	–	–	–	3+	3+	3+	3+
	Mucor racemosus	–	–	–	–	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+

The toxicity of the copper salts varies both with the specific nature of the salt as well as with the resistance of the test organisms. Although all the copper salts inhibited *Penicillium puberulum* and *Alternaria sp.* in concentrations of 0.5 per cent, *Aspergillus niger* was inhibited by cupric sulphate and cupric acetate in concentrations of 0.5 and 0.6 per cent, respectively.

Doran in 1923 (3) states that Clark found aspergilli to be more resistant to copper than penicillia, which agrees with the above-mentioned data.

The data demonstrate, first, that the concentration necessary to inhibit growth is far too strong for practical use and, second, that the marked variability in resistance of the three molds would make the use of such preparations very unreliable practically.

Mercury salts show much better mycostatic properties than copper salts, but the objection to their use in foods precludes any possibility of the trade acceptance of them.

It is entirely possible to prevent the growth of molds on fillers, flats and cases by impregnating them with contact mycostats, but the eggs would still mold if spores were present on their surfaces at the time of storage. The application of contact mycostats does not appear to offer much benefit in the control of molds on eggs and egg containers.

Tests on Compounds with High Vapor Pressures

Although a number of compounds having high vapor pressures was examined, a series of phenol derivatives, called Dowicides, manufactured by the Dow Chemical Company, was finally selected for intensive investigations. The compounds tested were as follows: sodium orthophenylphenate (Dowicide A), sodium 2, 4, 5 trichlorophenate (Dowicide B), sodium 2 chloro-orthophenylphenate (Dowicide C), sodium 2, 4, 5, 6 tetrachlorophenate (Dowicide F), and sodium pentachlorophenate (Dowicide G).

The mycostatic powers of those compounds were tested to determine the maximum mycostatic dilution. The compounds were tested on species of four genera of molds, as follows: *Aspergillus niger, Penicillium puberulum, Chaetomium globosum,* and *Mucor racemosus.* The data are presented in Table 10.

The mycostatic effect of the Dowicides on these four molds showed *Mucor racemosus* the most resistant with *Penicillium puberulum* next. The fact that these compounds prevented even the growth of *Mucor racemosus* in concentrations of 0.02 per cent indicates that this series of products is capable of preventing all molds encountered on eggs, because this mold is the most resistant one tested in these experiments.

To check further, 14 strains and species of penicillia were tested against these products. The data for sodium ortho-phenyl phenate and sodium 2 chloro-ortho-phenyl phenate are presented in Tables 11 and 12, respectively. The other compounds behaved similarly. The chlorinated phenols gave in every instance a higher mycostatic action. It was interesting to the authors to note that at the concentrations tested all the penicillia used gave the same results for each compound.

In all the tests presented, the mycostatic action has been measured by adding the mycostat to a nutrient medium. Under those conditions the mold had a very favorable opportunity for growth because of the rich nutrient medium. Furthermore, the cultures were incubated at room temperature, which is optimum for the development of the molds.

To simulate more nearly cold storage conditions the Dowicides were

TABLE 11. *The resistance of 14 cultures of penicillia to sodium orthophenylphenate.*

Organism	Per cent concentration of sodium orthophenylphenate					
	.2	.1	.02	.002	.0002	Control
P. puberulum—Strain 1	−	−	∓	+	+	3+
P. puberulum—Strain 2	−	−	∓	+	+	3+
P. puberulum—Strain 3	−	−	∓	+	+	3+
P. puberulum—Strain 4	−	−	∓	+	+	3+
P. roqueforti	−	−	∓	+	+	3+
P. verrucosum	−	−	∓	+	+	3+
P. casei	−	−	∓	+	+	3+
P. citrinum	−	−	∓	+	+	3+
P. crustosum	−	−	∓	+	+	3+
P. oxalicum	−	−	∓	+	+	3+
P. 131.1	−	−	∓	+	+	3+
P. flavo-glaucum	−	−	∓	+	+	3+
P. janthinellum	−	−	∓	+	+	3+
P. viridicatum	−	−	∓	+	+	3+

+ fair growth; ∓ scanty growth; − no growth.

applied to cases, fillers, and flats. Two-inch squares of cases, fillers, and flats were prepared. These were soaked for 5 minutes in the various dilutions of the phenol preparations and then placed individually in deep culture dishes. Subsequently they were seeded with a spore mixture of species of four different genera of molds, namely, *Aspergillus niger, Penicillium puberulum, Chaetomium globosum,* and *Mucor racemosus.* A piece of moist cotton was placed beneath each square in the deep culture dish. During the incubation period this cotton was kept moist to insure proper moisture conditions for the growth of the mold. Growth was measured by the macroscopic appearance of the mycelia on the materials tested.

This method of application of the mycostatic agents did not insure that the egg packing materials would absorb the same concentration of the mycostat as that in the solution applied, but it did give a comparable method of measurement of the compounds.

TABLE 12. *The resistance of 14 cultures of penicillia to sodium 2 chloroorthophenylphenate.*

Organism	Per cent concentration of sodium 2 chloroorthophenylphenate					
	.2	.1	.02	.002	.0002	Control
P. puberulum—Strain 1	−	−	−	+	+	3+
P. puberulum—Strain 2	−	−	−	+	+	3+
P. puberulum—Strain 3	−	−	−	+	+	3+
P. puberulum—Strain 4	−	−	−	+	+	3+
P. roqueforti	−	−	−	+	+	3+
P. verrucosum	−	−	−	+	+	3+
P. casei	−	−	−	+	+	3+
P. citrinum	−	−	−	+	+	3+
P. crustosum	−	−	−	+	+	3+
P. oxalicum	−	−	−	+	+	3+
P. 131.1	−	−	−	+	+	3+
P. flavo-glaucum	−	−	−	+	+	3+
P. janthinellum	−	−	−	+	+	3+
P. viridicatum	−	−	−	+	+	3+

3+ excellent growth; 2+ good growth; + fair growth; − no growth.

TABLE 13. *The mycostatic powers of egg-case materials impregnated with phenol derivatives.**

Mycostat	Medium	Per cent concentration of mycostat										
		2	1	0.1	.05	.03	.025	.02	.016	.014	.012	Control
Sodium orthophenylphenate (Dowicide A)	Wood..	−	−	3+	3+	3+	3+	3+	3+	3+	3+	3+
	Flat...	−	−	3+	3+	3+	3+	3+	3+	3+	3+	3+
	Filler..	−	−	3+	3+	3+	3+	3+	3+	3+	3+	3+
Sodium 2, 4, 5 trichlorophenate (Dowicide B)	Wood..	−	−	−	−	−	−	−	−	−	+	3+
	Flat...	−	−	−	+	+	+	+	2+	2+	2+	3+
	Filler..	−	−	−	+	+	+	+	2+	2+	2+	3+
Sodium 2 chloroorthophenylphenate (Dowicide C)	Wood..	−	−	−	−	−	−	−	+	+	+	3+
	Flat...	−	−	−	+	+	+	+	2+	2+	2+	3+
	Filler..	−	−	−	+	+	+	+	2+	2+	2+	3+
Sodium 2, 4, 5, 6 tetrachlorophenate (Dowicide F)	Wood..	−	−	−	−	−	2+	2+	2+	2+	2+	3+
	Flat...	−	−	+	2+	2+	2+	2+	2+	2+	2+	3+
	Filler..	−	−	+	2+	2+	2+	2+	2+	2+	2+	3+
Sodium pentachlorophenate (Dowicide G)	Wood..	−	−	+	+	+	3+	3+	3+	3+	3+	3+
	Flat...	−	+	+	+	+	3+	3+	3+	3+	3+	3+
	Filler..	−	+	+	+	+	3+	3+	3+	3+	3+	3+

*The materials were soaked 5 minutes in the mycostatic solutions.
3+ excellent growth; 2+ fair growth; + slight growth; − no growth.

The data from this study are presented in Table 13. The chlorophenols gave the best results.

In a second series of experiments, fillers and flats were impregnated with sodium 2, 4, 5 trichlorophenate (Dowicide B), sodium 2, 4, 5, 6 tetrachlorophenate (Dowicide F), and sodium pentachlorophenate (Dowicide G). The fillers and flats were analyzed and found to contain the following amounts:

Dowicide B ..... 0.81% dry weight
Dowicide F ..... 0.92% dry weight
Dowicide G ..... 0.86% dry weight

Two-inch squares were prepared and tested in the same manner as in the previous experiment. After 79 days incubation at 17° C. the test samples were examined. The data are presented in Table 14. All the treated fillers and flats were free from mold growth, whereas the untreated material was heavily contaminated with mold mycelia and spores.

The tests presented on the compounds showing vapor pressures indicate that all these compounds are markedly mycostatic. In the following experiments attempts were made to measure mycostatically the power of these compounds to give off vapor from the treated fillers and flats by placing untreated eggs contaminated with a mixture of mold spores in contact with the treated fillers and flats or in an enclosure containing the mycostats.

The tests were made as follows: two quart, wide-mouth Mason jars were used as containers. Four eggs were placed on a wire screen raised above the bottom of the fruit jar by a 2½ inch wooden block. The eggs were enclosed within a miniature "egg case" made from egg-case filler and flat material cut to fit the jar. Water was placed in the bottom of the jar to keep the atmosphere saturated with moisture.

TABLE 14. *Mycostatic powers of fillers and flats treated with chlorophenates on spore mixture of Penicillium puberulum, Aspergillus niger, Mucor racemosus, and Chaetomium globosum.*

Treatment	Concentration	Sample No.	Presence of mold
Sodium orthophenylphenate (Dowicide A)	0.81%	1.	−
		2.	−
		3.	−
		4.	−
Sodium 2, 4, 5, 6 tetrachlorophenate (Dowicide F)	0.92%	1.	−
		2.	−
		3.	−
		4.	−
Sodium pentachlorophenate (Dowicide G)	0.86%	1.	−
		2.	−
		3.	−
		4.	−
None	−	1.	4+
		2.	4+
		3.	4+
		4.	4+

All cases, fillers, flats, and eggs were sprayed with a heavy suspension of mold before being packed.

In the first series of tests, experiments were conducted to determine the best method of measuring mycostatic powers. Sodium 2 chloro-orthophenylphenate (Dowicide C) and sodium 2, 4, 5 trichlorophenate (Dowicide B) were used in three jars each, being applied as a dry powder, as a solution, and by impregnating the egg-case material. The data obtained after two months' storage at room temperature (Table 15) demonstrate that the application of the mycostat to the fillers and flats prevented entirely the growth of molds on both the egg-case material and the eggs. The vapor given off by the powder or the solution, however, was insufficient to carry to the eggs and fillers and flats a sufficient quantity to suppress growth. Impregnating the fillers and flats made these compounds mycostatic and also brought the emanating of vapors to the desired point of attack, namely, the egg surface which is, at few points, in direct contact with the mycostat in the fillers and flats.

TABLE 15. *Results of applying phenol derivatives by different methods.*

Mycostatic agent	Method of application	Amount of mold growth on eggs and packing materials
Sodium 2 chloroorthophenylphenate (Dowicide C)	Dry powder	Heavy growth of mold
	1-500 solution	Heavy growth of mold
	Packing materials impregnated	Slight amount of mold
Sodium 2, 4, 5 trichlorophenate (Dowicide B)	Dry powder	Moderate growth of mold
	1-500 solution	Moderate growth of mold
	Packing materials impregnated	No mold growth

The comparative value of the Dowicides was next tested under actual cold storage conditions by preparing Mason jars as in the previous experiment, using fillers, flats, and cases that had been soaked in various dilutions of the Dowicides. After the eggs were placed in the jars, a suspension of a spore mixture was sprayed over their surfaces. Duplicate jars were made in all tests. The jars were stored at a temperature of 34° F. for 84 days. The data are presented in Table 16. All the compounds tested (Dowicides A, C, F, and G) prevented the development of mold on the egg surface. The weak concentrations of Dowicides A, C, and G allowed a very slight development of mold at the contact point of the filler and the egg.

A similar experiment was performed using fillers and flats in which the exact amount of mycostat had been determined. These data are shown in Table 17. It will be observed from an examination of the

TABLE 16. *Mycostatic action of Dowicides A, C, F, and G treated fillers, flats, and cases on eggs stored for 84 days at 34° F. in saturated atmosphere contaminated with spore mixture.*

Treatment	Per cent concentration*	Presence of molds			
		Fillers	Flats	Cases	Eggs
None		4+	4+	4+	4+
Sodium orthophenylphenate (Dowicide A)	0.05	+	+	−	−
	0.07	+	+	−	−
	0.09	−	−	−	−
	0.1	−	−	−	−
	0.2	−	−	−	−
	0.3	−	−	−	−
Sodium 2 chloroorthophenylphenate (Dowicide) C	0.05	+	+	+	−
	0.07	+	+	−	−
	0.09	−	−	−	−
	0.1	+	+	−	−
	0.2	−	−	−	−
	0.3	∓	∓	−	−
	0.4	∓	∓	−	−
	0.6	−	−	−	−
	0.8	−	−	−	−
Sodium 2, 4, 5, 6 tetrachlorophenate (Dowicide F)	0.05	−	−	−	−
	0.07	−	−	−	−
	0.09	−	−	−	−
	0.1	−	−	−	−
	0.2	−	−	−	−
	0.3	−	−	−	−
	0.4	−	−	−	−
	0.6	−	−	−	−
	0.8	−	−	−	−
Sodium pentachlorophenate (Dowicide G)	0.5	+	+	−	+
	0.07	+	+	−	−
	0.09	+	+	−	+
	0.1	+	+	+	+
	0.2	+	+	−	−
	0.3	−	−	−	−
	0.6	−	−	−	−
	0.7	−	−	−	−
	0.8	−	−	−	−

*Fillers, flats, and cases soaked in concentrations indicated.

TABLE 17. *Mycostatic properties of Dowicides B, F, and G treated fillers and flats on eggs stored for 115 days at 34° F. in saturated atmosphere contaminated with spore mixture.*

Treatment	Per cent concentration*	Sample No.	Presence of molds	
			Fillers	Eggs
None	—	1.	4+	4+
		2.	4+	4+
		3.	4+	4+
		4.	4+	4+
Sodium 2, 4, 5 trichlorophenate (Dowicide B)	0.81	1.	—	—
		2.	—	—
		3.	—	—
		4.	—	—
Sodium 2, 4, 5, 6 tetrachlorophenate (Dowicide F)	0.92	1.	—	2+
		2.	—	+
		3.	—	+
		4.	—	+
Sodium pentachlorophenate (Dowicide G)	0.86	1.	—	—
		2.	—	∓
		3.	—	—
		4.	—	∓

*Concentration in per cent dry weight.

table that Dowicide B gave the best results. Using Dowicide G, a very slight amount of mold was found on two eggs out of the 16 eggs tested. Dowicide F allowed mold to develop on 12 of the 16 eggs.

The comparative mycostatic vapor concentration of the Dowicides at a temperature of 34° F. compares very favorably with the vapor pressures of these compounds at temperatures above 100° C. In Fig. 1† are presented the vapor pressures of Dowicides 1, 2, 3, 6 and 7 which are the phenols from which the sodium salts, Dowicides A, B, C, F, and G, respectively, are prepared.

An analysis of the data on the laboratory studies indicates that Dowicides B and G should be the most satisfactory for practical application for the impregnation of fillers and flats and the dipping of cases. Inasmuch as the Dowicide G is the more stable compound, it seems that this compound should be the best because it would continue to act for a longer period in the fillers and flats, and its mycostatic action is only slightly less than Dowicide B. Furthermore, the cost of the treatment would be considerably less.

†This figure was prepared by the Dow Chemical Company. It will be noted that Dowicide B has the highest vapor pressure and also gives the best results. This is in agreement with the mycological results obtained.

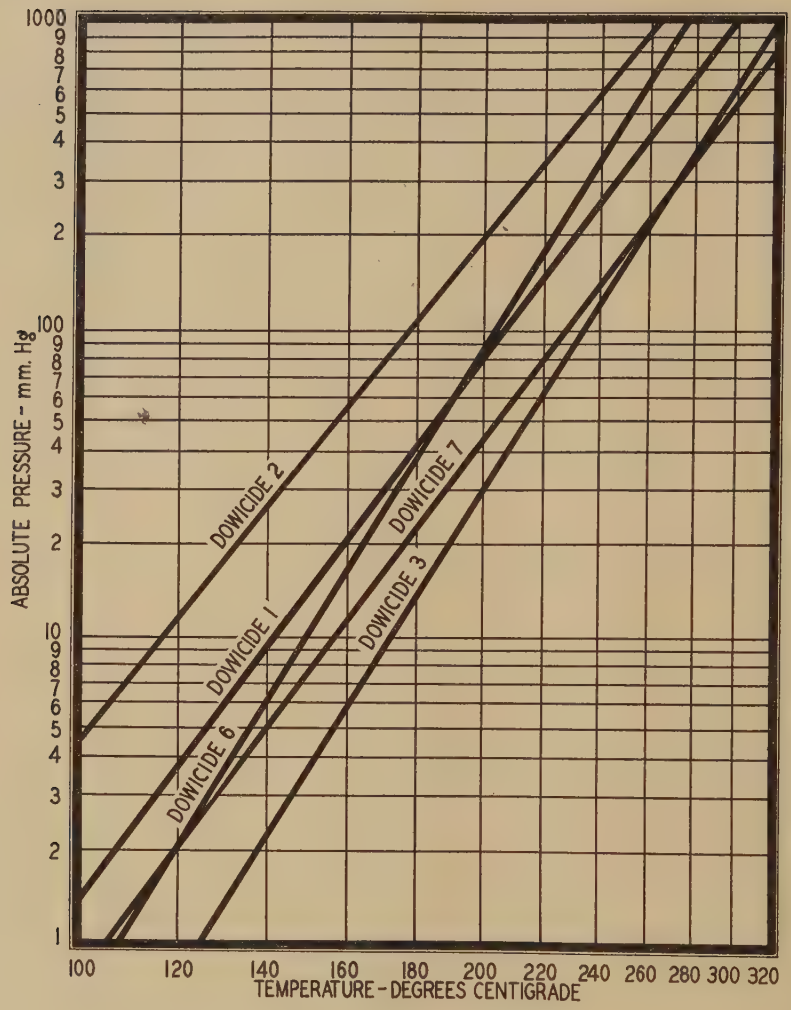

Fig. 1. Vapor pressure of Dowicides.

PART III.

PRACTICAL TESTS ON MYCOSTATS UNDER COMMERCIAL CONDITIONS WITH HIGH AND LOW HUMIDITIES

With the laboratory tests completed, a field test was made using five half-cases of eggs. Four half-cases of eggs were sprayed heavily with a concentrated suspension of spores obtained from eighteen of the species of molds isolated from eggs. In each instance the molds were applied as each layer of eggs was placed in the cases. The fifth half-case was kept as a control. Three of the wooden cases were moistened with a 0.1-per cent solution of the mycostat. Dowicides B, C, and G were used, one for each of the three cases, and a 0.1-per cent solution of the desired mycostat was sprayed lightly over the eggs. One of the cases seeded with mold spores was treated with water in the same manner without the addition of the mycostat. The fifth half-case, the control, was treated with water but no spores were added.

The cases of eggs were placed in cold storage for five months when the eggs were removed and examined carefully for mold formation. The data are presented in Table 18. The data show that when Dowicide C was applied, only 2.5 per cent of the eggs showed signs of mold formation and all of the fillers and flats were mold-free. When Dowicide B was used, 29.1 per cent of the eggs were molded and two fillers showed slight mold formation. With Dowicide G treatment, 28.3 per cent of the eggs, one filler and one flat, were slightly molded. The water-treated set, however, showed 94.1 per cent of the eggs, four fillers and two flats, contaminated with molds. The control case in which the eggs were not wet excessively but sprayed with mold spores showed only 26.6 per cent of the eggs and two fillers contaminated. Although the mycostat agents were applied to the eggs and egg-packing materials as a light spray and the spore load of the eggs was exceedingly heavy, the phenol preparations were able to suppress materially the development of molds on the eggs and egg packing materials.

In the above-mentioned experiments, the eggs were stored at a

TABLE 18. *The mycostatic powers of phenol preparations applied to eggs in cold storage.*

Mycostat	Treated with molds	Moldy eggs		Number moldy fillers	Number moldy flats
		Number	Per cent		
Water	−	32	26.6	2	0
Water	+	113	94.1	4	2
Sodium 2, 4, 5 trichlorophenate (Dowicide B)	+	35	29.1	2	0
Sodium 2 chlorophenylphenate (Dowicide C)	+	3	2.5	0	0
Sodium pentachlorophenate (Dowicide G)	+	46	38.3	1	1

low relative humidity, approximately 85 per cent, but the cases, fillers, and flats were very wet at the time of storage. Because the humidity was not accurately controlled and the amount of moisture to which the

TABLE 19. *The mycostatic powers of phenol preparations applied to eggs in cold storage at a relative humidity of 98 per cent and temperature of 32° F. for three months.*

Serial No.	Solution Used	Treatment applied to			Kind of eggs	No. of eggs	Appearance of mold[3]		
		Cases	Fillers and flats	Eggs			Cases	Fillers and flats	Eggs
No treatment									
21-A..	None.....	No	No	No	Ord.[1]	179	No	No	No
12-A..	None.....	No	No	No	Pep.[2]	177	No*	No	3
Spare.	None.....	No	No	No	Pep.	123	No	No	3
Treatment with water									
21-B..	Water.....	Yes	Yes	No	Ord.	162	10	10	10
12-B..	Water.....	Yes	Yes	No	Pep.	171	No*	2	4
Treatment—Dowicide A									
1....	1% Dow. A....	Yes	Yes	No	Ord.	169	No	1	1
2....	1% Dow. A....	Yes	Yes	Yes	Ord.	172	No	1	1
3....	0.1% Dow. A..	No	Yes	No	Pep.	180	8	1	2
4....	0.1% Dow. A..	Yes	Yes	No	Pep.	179	1	1	1
5....	0.1% Dow. A..	Yes	Yes	Yes	Ord.	177	1	3	1
6....	0.05% Dow. A.	No	Yes	No	Ord.	172	6	10	10
Treatment—Dowicide B									
7....	0.5% Dow. B..	Yes	Yes	Yes	Ord.	180	No	No	No
8....	0.5% Dow. B..	Yes	Yes	No	Ord.	180	No	1	1
9....	0.2% Dow. B..	Yes	Yes	No	Ord.	180	No	2	2
10....	0.1% Dow. B..	Yes	Yes	No	Ord.	180	No	4	3
11....	0.1% Dow. B..	Yes	Yes	No	Ord.	324	No*	3	3
Treatment—Dowicide C									
13....	0.5% Dow. C..	Yes	Yes	Yes	Ord.	177	10	10	6
14....	0.5% Dow. C..	Yes	Yes	No	Ord.	179	10	4	2
15....	0.2% Dow. C..	Yes	Yes	No	Ord.	139	8	2	4
16....	0.1% Dow. C..	Yes	Yes	No	Ord.	180	10	6	6
17....	0.1% Dow. C..	Yes	Yes	No	Ord.	177	No*	2	2
Treatment—Dowicide F									
19....	1% Dow. F....	Yes	Yes	No	Ord.	174	No	5	5
Treatment—Dowicide G									
20....	1% Dow. G....	Yes	Yes	No	Pep.	164	No	3	8
Treatment—Azochlorimide									
18....	.....	Yes	Yes	No	Ord.	172	8*	8	8

*Sitka spruce case—all other tupelo gum or cottonwood.
(1) Ord.—ordinary run storage eggs.
(2) Pep.—Pacific Egg Producer oil treated eggs.
(3) Heavy mold attack is scored 10.

eggs were exposed was uncertain, better storage conditions were sought for succeeding experiments.

Humidity is such a primary factor in the development of molds in cold storage, however, that it was deemed necessary to test the various mycostats under conditions of controlled humidity much higher than is ever used in cold storage houses where eggs are stored.

Commercially packed eggs were stored in an experimental room* at relative humidities of 92-100 per cent at a temperature of 32° F. For comparative purposes, eggs were also stored at relative humidities of 88 per cent in a regular egg storage room. Solutions of various mycostats were sprayed over the eggs and FILLERS, FLATS, AND CASES WERE SOAKED in the solution of the mycostats. Varying concentrations of the various compounds were used. At the end of three months, examinations were made for mold formation.

The data for relative humidities are presented in Table 19. It will be noted that the eggs placed in cases in which no treatment was applied presented a more satisfactory appearance than did any of the treated eggs. These data would seem to indicate that treatment had caused an increase in spoilage rather than a decrease. As a matter of fact, this was true at the end of the three months' storage period. This was likely due to the comparatively lower humidity in these cases as compared with that of the others where the fillers and flats were wet at the time the eggs were stored. At the end of six months' storage these eggs would be heavily contaminated.

The eggs stored in fillers and flats which were wet but not treated with mycostats were heavily contaminated at the end of three months' storage. The oil-treated eggs stored in sitka spruce cases and white fillers and flats which repel water, having much less humidity, showed much less mold than did the ordinary eggs stored in fillers and flats which readily absorb water when dipped. All of these eggs at the end of six months' storage would be heavily contaminated.

The eggs treated with Dowicide B were in the best condition at the end of three months' storage. Dowicides A, C, F, and G were fairly effective but did allow some development of molds in three months.

The conditions that existed in the storage room at relative humidities of 95-100 per cent were highly impractical. The cases were so badly warped that the cases were completely ruined. The fillers and flats were so moist that it was impossible to lift them from the cases without tearing them. Under such conditions, moisture concentrations of the cases, fillers, and flats was optimum for the growth of fungi.

The extreme conditions were planned purposely to produce as nearly as possible optimum conditions for mold development at 32° F. so that the mycostats would be tested under the most severe situations. If molds can be suppressed in such an optimum environment, then unquestionably the mycostats would prevent mold development under the most severe conditions that might be experienced under commercial practice.

To show the influence of humidity in the development of molds, a similar set of eggs was stored for three months at a relative humidity of 88 per cent. An examination of Table 20 will show that practically

*Through the courtesy of the U. S. Cold Storage Company of Chicago, a storage room with high relative humidity was made available for these studies.

TABLE 20. *The mycostatic powers of phenol preparations applied to eggs in cold storage at a relative humidity of 88 per cent and temperature of 32° F. for three months..*

Serial No.	Solution Used	Treatment applied to			Kind of eggs	No. of eggs	Appearance of mold[3]		
		Cases	Fillers and flats	Eggs			Cases	Fillers and flats	Eggs
No treatment									
106	None	No	No	No	Ord.[1]	36	No	No	No
107	None	No	No	No	Pep.[2]	36	No	No	No
Treatment—Dowicide A									
112	1% Dow. A	No	Yes	No	Ord.	36	No	No	No
113	0.05% Dow. A.	No	Yes	No	Ord.	36	No	8	8
Treatment—Dowicide B									
108	0.5% Dow. B.	No	Yes	No	Ord.	36	No	No	No
114	0.1% Dow. B.	No	Yes	No	Ord.	36	No	No	No
115	0.1% Dow. B.	No	Yes	No	Pep.	36	No	No	No
Treatment—Dowicide C									
110	0.5% Dow. C.	No	Yes	No	Ord.	36	No	No	No
116	0.1% Dow. C.	No	Yes	No	Ord.	36	No	No	No
Treatment—Dowicide G									
109	1% Dow. G.	No	Yes	No	Ord.	36	No	No	No

(1) Ord.—ordinary run storage eggs.
(2) Pep.—Pacific Egg Producer oil treated eggs.
(3) Heavy mold attack is scored 10.

no molds appear on any eggs, fillers, or flats after three months' storage. Even at the end of six months' storage mold failed to appear even on the control cases of eggs.

In the experiments represented by the data in Tables 19 and 20 the exact amounts of mycostat in the fillers and flats were not known, so additional experiments were conducted using known quantities of the chemical agents. A half-case of eggs each was stored in fillers and flats which had been treated with Dowicides B, F, and G, respectively. The concentrations used are as follows:

Dowicide B ..... 0.81 per cent dry weight
Dowicide F ..... 0.92 per cent dry weight
Dowicide G ..... 0.86 per cent dry weight

At the end of the eight months' storage at 32° F., the eggs in the treated fillers and flats were without exception free from molds, whereas the eggs, fillers, and flats in the control case were badly molded. The relative humidity during this experiment ranged from 92-95 per cent.

In another experiment eggs were stored in fillers and flats which were impregnated with Dowicide G at the time of manufacture. These fillers and flats averaged from 0.3 to 0.4 per cent pentachlorophenol. Eggs in treated fillers and flats have failed to develop mold.

TABLE 21. *Egg examination for odor and taste. Eggs stored in fillers and flats treated with Dowicides B, F, and G. Eggs placed in storage at U. S. Cold Storage Plant, Chicago, Ill., Oct. 28, 1938. Eggs examined Feb. 11, 1939.*

Treatment	Egg No.	Scores*		Combined comments
		D. Grantham	R. Griswold	
Control	4	3.5	4	Ordinary storage egg taste.
	8	4.0	4	Ordinary storage egg taste.
	12	4.5	5	Slight storage egg taste.
	Average	4.0	3.3	
Dowicide B	1	4	4	Cracked before cooking.
	5	4	4.5	
	9	4	4.5	
	Average	4	4.3	Less storage taste—some odor.
Dowicide G	2	2.5	3	Spoiled smell—not good flavor.
	6	4.0	4	
	10	4.5	4	
	Average	3.7	3.7	
Dowicide F	3	3	4	Off-taste.
	7	3	4	Storage odor and taste.
	11	3	4.5	
	Average	3	4.2	

Remarks: All eggs with possible exception of No. 12 tasted and looked like storage eggs. No chemical taste with possible exception of No. 8 which may have been due to spoon.
*Scoring: 7—excellent; 6—very good; 5—good; 4—medium; 3—fair; 2—poor; 1—very poor.

The addition of chemical agents with high vapor pressures to fillers and flats would appear to be poor practice inasmuch as eggs absorb any odor in the storage room. There are grave dangers that such practice would impart a chemical taste and odor to the egg.

To ascertain whether Dowicides B, F, and G would impart flavor and odor to the eggs, eggs were stored in fillers and flats impregnated with approximately 0.8 per cent dry weight of Dowicides B, F, and G, respectively. A half-case of each was stored Oct. 29, 1938. On Feb. 11, 1939 eggs were removed and submitted to Ruth M. Griswold of the Home Economics Department of Michigan State College for organoleptic tests. The results are presented in Table 21. Eggs were also submitted to Mrs. Kathryn Niles of the American Institute of Poultry Industries. Their reports show that even with high concentrations of the Dowicides (0.8 per cent dry weight) it was impossible to detect any significant differences in egg quality.

Eggs were also stored in a number of cold storage houses, using fillers and flats treated with Dowicide G (0.4 per cent dry weight). These eggs had been stored for approximately six months when they were examined for quality. These eggs were stored in regular cold storage rooms. Representative results are shown in Tables 22 and 23 of eggs stored in three storage houses. In each case the eggs in treated cases were given slightly higher scores than those stored in untreated cases.

The data show conclusively that the introduction of Dowicides in quantities as high as 0.8 per cent had no deleterious effects upon egg quality as represented by taste and odor.

TABLE 22. *Egg examination for odor and taste. Eggs stored in fillers and flats treated with 0.4 per cent Dowicide G. Eggs stored at Central Storage Company, Chicago, Ill., April 10, 1939. Eggs examined Sept. 29, 1939.*

Treatment	Egg No.	Scores*		Combined comments
		M. Wharton	R. Griswold	
DEARBORN STREET PLANT				
Control	4	3	3.5	Off-flavor—slight.
	5	4.5	6	
	12	4	4	Slight stale odor.
	17	4	4	Bad taste and odor.
	21	2	4	Metallic flavor, slightly stale.
	24	4	3	Stale odor—off-flavor.
	30	3	3	Stale odor—Chem. Metallic taste.
	32	3	2	Stale odor and flavor—cardboard taste.
	35	4	4	Slight stale odor.
	38	2	2	Distinct taste and odor—not a good egg.
	41	5	5	
	44	4.5	5	
	Average	3.66	3.71	
Dowicide G	2	4	4	
	8	4	4	Slight odor.
	11	5	5	
	15	4	3	Slight stale odor—off-flavor.
	16	4.5	5.5	Slight stale odor.
	22	3	4	Fairly fresh.
	28	3	3	Stale odor and flavor—cardboard taste.
	31	4.5	5	
	34	5.	3.5	Unpleasant after-taste.
	37	3	2	Slight stale odor and taste.
	45	4.5	5	
	48	4.5	4	Slight after-taste.
	Average	4.08	4.00	
SANGAMON STREET PLANT				
Ozone Treatment	6	5	5	
	7	5	5	
	13	4	4	Slight stale odor.
	14	4	4.5	Slight stale odor.
	19	2.5	3.5	Slightly stale—off-flavor.
	20	5	4.5	
	27	3	2	Stale odor—metallic flavor.
	36	5.5	5.5	
	39	5	4	Slightly off-flavor.
	42	4	4	Slight stale odor.
	46	5.5	5	
	10	5	5	
	Average	4.46	4.33	
Dowicide G	1	4	6	Very slight stale odor and taste.
	3	4	4	Almost like fresh egg.
	9	5.5	6	
	18	5.5	5.5	
	23	4	5	Fairly fresh.
	25	3	5	Slight odor—sweet taste.
	29	5	5.5	
	33	5.5	4	Stale odor—off-flavor.
	40	2	3	Stale odor—metallic flavor.
	43	4.5	4	
	47	5	6	
	Average	4.42	4.75	

*Scoring: 7—excellent; 6—very good; 5—good; 4—medium; 3—fair; 2—poor; 1—very poor.

TABLE 23. *Egg examination for odor and taste. Eggs stored in fillers and flats treated with 0.4 per cent Dowicide G. Eggs stored at U. S. Cold Storage, Detroit, Mich., March 29, 1939. Eggs examined Oct. 17, 1939.*

Treatment	Egg No.	Scores*		Combined comments
		R. Griswold	H. Jimmerson	
Control	1	2.5	1	Metallic flavor.
	2	1.5	2	
	4	2	1.5	
	7	2.5	1.5	
	9	3	3	
	10	2	2.5	Bitter after-taste.
	11	1	2	Bad off-taste.
	13	2	3	
	17	4	4	
	20	2	4	Bad taste and odor.
	21	3	3	Off-taste.
	22	3	2.5	
	Average	2.29	2.50	
Dowicide G	3	1	1	
	5	3	1.5	
	6	1	1	
	8	3.5	5	
	12	1	2	Old straw taste.
	14	3	1	
	15	3	4	
	16	2	3	
	18	4	3	
	19	3	3	
	23	2	3	Off-taste.
	24	4	3	
	Average	2.54	2.54	

*Scoring: 7—excellent; 6—very good; 5—good; 4—medium; 3—fair; 2—poor; 1—very poor.

DISCUSSION

It has been demonstrated in the data submitted that the molds found on eggs and egg case materials are extremely common molds which are not peculiar to cold storage rooms. The fact that the same molds are common to fresh eggs and even to new egg packing materials means that sanitary handling of the eggs, although desirable, does not in any sense preclude the development of molds in the egg package during cold storage.

It is obvious that the elimination of moisture from the egg surfaces and from the fillers and flats would prevent the development of mold mycelia, but to maintain such conditions would mean a loss of moisture by the eggs. It has been common practice to maintain cold storage rooms at a relative humidity of 85 to 88 per cent to prevent mold growth. Occasionally with these low humidities molds sometimes appear, particularly if eggs are held longer than the usual storage period. If humidities are held to 90 per cent or above, molds will appear after four or five months' storage.

The only means of suppressing molds appears to be either a destruction of all molds on the eggs and the egg package or the suppression of growth by means of chemical agents. To destroy the molds is highly impractical but the introduction of chemical agents into the egg package is feasible.

In the studies presented, it has been demonstrated both in the laboratory and in commercial practice that mycostats without vapor pressure, although they may localize mold growths on an egg to its own compartment in the case, do not prevent the germination and growth of mold spores found on the eggshell. To prevent such development, the mycostats must have at least a low vapor pressure to envelope the egg in a mycostatic vapor.

Although many compounds capable of yielding mycostatic vapors were tested, only one, sodium pentachlorophenate (Dowicide G) was found entirely satisfactory. This compound, when introduced into the fillers and flats in concentrations of 0.4 per cent or more suppressed the growth of molds of all types not only on the egg package material but also on the egg surfaces. The mycostatic activity was sufficient even to suppress the molds at relative humidities much higher than could be maintained practically in the cold storage room. The compound does not impart any taste or odor to the egg during storage.

The ideal substance would be one (a) with maximum toxicity toward the fungi common to eggs, (b) available at low cost, (c) having no odor, and (d) showing some but relatively low vapor pressure. The compound should preserve the latter characteristic because egg packing materials are used for more than one season, and the compound should be retained for the life of the packing material.

Dowicide G can be added to the paper stock during manufacture at a cost so small that the added cost per set of fillers and flats is only a fraction of a cent. Although fillers and flats could be sprayed with a weak solution at the time of use, the former method of application (during manufacture) is more practical because of uniformity of application. Furthermore, to be of full value, all fillers and flats must be treated so that the humidity of the cold storage room can be raised. If only part of the fillers and flats were treated, the humidity would have to be maintained at the usual lower level to protect these eggs stored in the untreated fillers and flats. To be successfully applied, all fillers and flats, irrespective of whether they are to be used for long or short storage, should be treated.

The treatment of the case to prevent mold developments is of little importance so far as the egg is concerned if the fillers and flats have been treated. However, cases free from mold spot present a better appearance, which is important. It was found in this study that the dipping of egg cases in a 1-per cent solution of Dowicide G prevented the growth of all molds. The most satisfactory method of applying the mycostat would be at the time of manufacture. At the present time many mills are treating lumber by this process to prevent sap stain and decay. Cases made of Sitka spruce are naturally resistant to mold growth, but gum and cottonwood are very susceptible. The latter woods should be treated.

SUMMARY

1. Representatives of ten genera were repeatedly isolated from eggs and egg packing material. The majority isolated belong to the genus Penicillium, but no one species predominated.

2. Not all of the molds penetrated into the interior of the egg under cold storage conditions. Only two genera were isolated from the interior of the shell. These consisted of 25 species of Penicillium and four strains of Hormodendron.

3. Two groups of compounds were tested for their value as mycostatic agents to be used to control mold development on eggs in cold storage. The first group consisted of compounds having little or no vapor tension which act by contact with the mold spore or mycelium. The second group were compounds with a vapor pressure sufficient to carry the inhibiting action to the mold without direct contact with the mold spore or mycelium.

The compounds with relatively high vapor pressure proved to be the best mycostatic agents both in the laboratory tests and under commercial cold storage conditions.

4. Of all the compounds tested, the following proved to be the most effective in controlling the development of mold on eggs in cold storage: sodium 2, 4, 5 trichlorophenate (Dowicide B), sodium 2, 4, 5, 6 tetrachlorophenate (Dowicide F), and sodium pentachlorophenate (Dowicide G).

Although sodium 2, 4, 5, trichlorophenate (Dowicide B) was the best mycostatic agent under the drastic experimental conditions used, sodium pentachlorophenate (Dowicide G) was found to be the most satisfactory mycostatic agent under ordinary commercial conditions. It was also better from the standpoint of low cost, slight odor, and a relatively low vapor pressure.

5. Eggs stored in flats and fillers treated with these phenol preparations were no different in odor and taste than the eggs stored under identical conditions in ordinary flats and fillers.

6. The most effective method of applying the mycostat was by impregnating the fillers, flats, and cases.

LITERATURE CITED

1. Anonymous.
1936. Control of odors and molds by ozone and ionized oxygen. Food Industries *8:* 307.

2. Bidault.
1922. (Molds on cold storage meat.) Bul. Soc. Sci. Hyg. Aliment. 10:12-25. (Abstract) Experiment Station Record *48:* 258.

3. Doran, W. L.
1923. Toxicity studies with some copper fungicides. Phytopath. *13:* 532-542. 9 tables.

4. Ewell, Arthur W.
1936. The use of ozone in cold storage plants. Ice and Refrigeration. *91:* 295-6.

5. Ewell, Arthur W.
1938. Present use and future prospects of ozone in food storage. Food Research *3:* 101-108.

6. James, L. H., and T. L. Swenson.
1929. A preliminary study of pin-spot molding of shell eggs in cold storage. U. S. Egg and Poultry Magazine *25* (12) : 47-49. 3 figs., 1 table.

7. Monvoisin, A.
1922. (Mold formation in meat kept in cold storage.) Recueil de Medecine Veterinaire *98:* 149-161. 3 figs.

8. Moran, T.
1938. Gas storage of eggs. The U. S. Egg and Poultry Magazine *44:* 304.

9. Naumov, N.
1935. Determination of Mucorales. Second revised edition, Botanical Institute of Academy of Sciences, U. S. S. R. 136 pp., 49 figs. Moscow and Leningrad (Russian). Unpublished translation by E. A. Bessey.

10. Pennington, Mary E.
1938. Roving thoughts on cold storage of eggs. U. S. Poultry and Egg Magazine *44:* 242.

11. Thom, Charles.
1930. The Penicillia. xiii + 644 pp. 98 figs. Williams and Wilkins Co., Baltimore, Md.

12. Thom, Charles, and Margaret B. Church.
1926. The Aspergilli. ix + 272 pp., 4 plates, 14 figs. Williams and Wilkins Co., Baltimore, Md.

13. Weston, W. A. R. D., and E. T. Halnan.
1927. Black spot of eggs. Poultry Science *6:* 251-258.

14. Wright, A. M.
1923. Molds on frozen meat. Journal of the Society of Chemical Industry 42: pt. 2, Trans., 488.

15. Yesair, John.
1928. The action of disinfectants on molds. Thesis, University of Chicago, Chicago, Ill. (Unpublished).

11-40: 5M